DEVELOPMENT OF BIOGRANULES USING SELECTED MIX CULTURE OF DECOLOURISING BACTERIA FOR TREATMENT OF TEXTILE WASTEWATER

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Specially dedicated to my family members

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ABSTRACT

Biological treatment of textile wastewater using granules involves the application of self-immobilisation of high density biomass under intermittent facultative anaerobic and aerobic system. Since granules are dense and have high settling velocity, high density of active biomass to be retained without being washed out, minimising previous problems of using suspended biomass in treating textile wastewater. The use of synthetic wastewater containing single or several combinations of dyes for the development of granules has been widely studied. However, little has been reported on the development of granules using more complex and toxic real textile wastewater. Hence, there is a need to develop granules that are well adapted to real textile wastewater in order to improve the treatment efficiency. In this study, granules consisting of bio-augmented consortium of four locally isolated decolourising bacteria were successfully developed under intermittent facultative anaerobic-aerobic system. Sludge was added as seeding agent in a single 1 L SBR reactor at hydraulic retention time (HRT) of 6 h. The 16S rDNA molecular analysis showed that ZK1 (JQ773350), ZK2 (JQ773351), ZB1 (JF742762) and ZB2 (JF742761) were closely related to *Bacillus pumilus*, *Bacillus cereus*, *Brevibacillus panacihumi* and *Lysinibacillus fusiformis* respectively. After 112 days, the size of the granules reached 3.3 ± 1 mm and were dark grey in colour, with integrity coefficient of 25 ± 2, settling velocity of 56 ± 5 m h^{-1} and sludge volume index (SVI) of 35 ± 5.5 mL g^{-1}. Biomass concentration was 13 ± 0.8 g L^{-1} and 11 ± 0.6 g L^{-1} for MLSS and MLVSS respectively. In general, the developed granules showed good removal for colour (70 %; initial ADMI values ranging from 500 to 2000) and COD (53 %; initial values ranging from 400 to 1,500 mg L^{-1}) at HRT of 24 h with intermittent facultative anaerobic (18 h) and aerobic (6 h).

ABSTRAK

Rawatan biologi air sisa tekstil dengan granul melibatkan pengumpalan biojisim berkepekatan tinggi dalam sistem olahan berselang seli fakultatif anaerobik dan aerobik. Dengan ketumpatan dan halaju enapan yang tinggi, granul yang terdiri daripada biojisim aktif yang berkepekatan tinggi dapat dikekalkan dalam reactor dan ini mengurangkan masalah penggunaan biojisim terampai dalam rawatan sebelum ini. Air sisa sintetik dengan satu atau kombinasi perwarna telah diguna secara meluas untuk pembentukan granul. Namun, pembentukan granul dengan air sisa tekstil mentah yang lebih kompleks dan toksik jarang dilaporkan. Oleh itu, keadaan ini menjanakan permintaan untuk pembentukan granul yang bersesuaian dengan air sisa tekstil mentah dalam meningkatkan prestasi rawatan., Granul dalam kajian ini terdiri daripada kombinasi empat bacteria penyahwarna diasing daripada kilang tekstil tempatan terbentuk dalam sistem olahan berselang seli fakultatif anaerobik dan aerobik. Enapcemar ditambahkan dalam reactor penjujukan berkelompok 1 L dengan masa tahanan hidraul selama 6 jam. Analisis penjujukan telah mengenal pasti ZK1 (JQ773350), ZK2 (JQ773351), ZB1 (JF742762) and ZB2 (JF742761) masing-masing berkait rapat dengan *Bacillus pumilus*, *Bacillus cereus*, *Lysinibacillus fusiformis* dan *Brevibacillus panacihumi*. Selepas 112 hari, granul terbentuk mencapai saiz 3.3 ± 1 mm dan berwarna kelabu gelap, dengan koefisien intergriti 25 ± 2, halaju enapan purata 56 ± 5 m j^{-1} dan indeks halaju enapan (SVI) 35 ± 5.5 mL g^{-1}. Kepekatan biojisim dalam campuran pepejal terampai (MLSS) dan campuran pepejal terampai sejatan (MLVSS) adalah masing-masing 13 ± 0.8 g L^{-1} dan 11 ± 0.6 g L^{-1}. Secara umumnya, granul yang terbentuk berkeupayaan menyahwarna (70 %; nilai awal Indeks Pembuatan Pewarna Amerika: 500 hingga 2000) dan penyingkiran COD (53 %; nilai awal: 400 hingga 1,500 mg L^{-1}) pada masa tahanan hidraul 24 jam dengan sistem olahan berselang seli fakultatif anaerobik (18 jam) dan aerobic (6 jam).

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16S rRNA	-	16 subunit ribosomal ribonucleic acid
ADMI	-	American Dye Manufacturing Index
Ag_2SO_4	-	Silver Sulfate
AOP	-	Advanced Oxidation Processes
APHA	-	American Public Health Association
BLASTn	-	Basic local alignment search tool
BOD	-	Biological Oxygen Demand
$C_6H_4\ (CH_3)_2$	-	Xylene
CaCl	-	Calcium Chloride
CAg	-	Co-aggregation
COD	-	Chemical Oxygen Demand
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen (mg/L)
EDTA	-	Ethylene diamine tetraacetic acid
EPS	-	Extracellular polymeric substances
FESEM	-	Field-Emission Scanning Electron Microscope
FISH	-	Hybridisation
H_2O_2	-	Hydrogen Peroxide
$HgSO_4$	-	Mercury (II) Sulfate
HRT	-	Hydraulic Retention Time
IC	-	Integrity Coefficient
$K_2Cr_2O_7$	-	Potassium Dichromate
K_2HPO_4	-	Dipotassium hydrogen phosphate
KH_2PO_4	-	Potassium dihydrogen phosphate
$MgSO_4$	-	Magnesium Chloride

MLSS	-	Mixed-Liquor Suspended Solid
MLVSS	-	Mixed-Liquor Volatile Suspended Solid
NA	-	Nutrient agar
NB	-	Nutrient broth
NCBI	-	National Center of Biotechnology Information
OLR	-	Organic loading rate (mg L^{-1} day^{-1}/kg m^{-3} day^{-1})
PCR	-	Polymerase chain reaction
PLCs	-	Programmable Logic Controllers
PS	-	Polysaccharide
RG	-	Residual granules (mg)
SBR	-	Sequencing Batch Reactor
SDS	-	Sodium dodecylsulfate
SG	-	Settled granules (mg)
SHb	-	Surface hydrophobicity
SRT	-	Sludge retention time (day)
SVI	-	Sludge Volume Index
TOC	-	Total Organic Carbon
TSS	-	Total Suspended Solid
UASB	-	Up flow Anaerobic Sludge Blanket
USEPA	-	United States Environmental Protection Agency
VER	-	Volumetric exchange rate
WWTPs	-	Wastewater Treatment Plants

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Textile industry is a major source of wastewater as it uses large amounts of water in its preparation and dyeing processes. It accounts for 22 % of total industrial wastewater produced in Malaysia (Rakmi, 1993). A survey of the Malaysian textile industry has revealed that the volume of wastewater generated by dyeing and finishing operations ranged from 73 to 167 m^3 per ton of product (Lin and Peng, 1996).

In general, textile wastewater is highly coloured due to persistent organics together with various pollutants such as chloride, ammonia, organic nitrogen, nitrate, sulphate, phosphate and heavy metals such as Fe, Zn, Cu and Pb. Synthetic dyes that have been extensively used in the textile dyeing industries are azo dyes due to its economical, stability and variations in colour compared to natural dyes (Griffiths, 1984). When textile is being processed, low efficiency in dyeing may cause huge volume of dyestuff to flow into the effluent, ending up in the environment (McMullan *et. al.*, 2001). The dyes are designed to be stable, thus are resistant to the microbial and physiochemical attack, and therefore, difficult to eliminate. The biological treatment process *via* the facultative anaerobic-aerobic treatment system that was developed from previous studies shows good potential application for the local textile industries as current treatment practice at most textile plants fail to meet the discharge limit while producing a lot of toxic sludge.

Over the years, the biological treatment of azo dyes has become increasingly important due to its eco friendly and economical features compared to the physical and/or chemical treatment processes. In biological treatment, bacterial decolourization of azo dyes involves azo reduction and/or desulphonation. The decolourisation was due to reductive cleavage of azo bond(s) catalysed by enzymes such as flavin reductase and quinone reductase (Russ *et al.,* 2001). In addition, desulphonation that results in destabilization of benzene ring structure is responsible for azo bonds decolourisation (Kertesz and Wietek, 2001).

The biological treatment utilises bacterial culture is either in suspension or immobilized on support materials. Comparatively, immobilised cells or biofilms were reported to be more efficient than that of suspended cells (Pearce *et al*., 2003). Granules have regular and rounded surface with denser and more compact structure if compared to the fluffy, irregular and loose structure of conventional bioflocs. With hard and dense microbial constitutions that have been successfully developed in the past few years, granular compactness along with the settling velocity is greater compared to traditional bio flocs (Beun *et al*., 1999).

Granulation as one of current immobilisation mechanism can therefore retain higher density of biomasses in reactor with this excellent settling ability. These high levels of active biomass with broad microbial diversity within the granular system lead to improvement of the treatment capacity and efficiency (McHugh *et al.,* 2003). Overall, granules are known with high settling velocity, COD removal, specific gravity, physical strength, integrity coefficient, hydrophobicity values and low sludge volume index (Guven, 2004). Besides, granules have also been proven to have the ability to treat high organic loading rate (OLR) wastewaters as the result of high concentration biomass retention within relatively miniature system that used up limited land area. Hence, this contributes to the establishment of small footprint and economical wastewater treatment plants (Hulshoff Pol *et al.*, 2004 and Etterer, 2006).

Furthermore, the effectiveness of granulation treatment was further enhanced by the use of augmented mixed cultures in terms of biodegradation rates and mineralisation. Due to self complemented co-metabolic activities within a microbial community, microbial consortia are able to collectively carry out biodegradation tasks which are next to impossible for individual strains. In addition, these specialised bacteria that had been acclimatised in the textile wastewater are probably more capable of treating the raw textile wastewater in terms of colour and COD removal. Therefore, granulation using augmented and specially adapted mix culture in a combined facultative anaerobic-aerobic system used in sequencing batch reactor (SBR) would provide both reducing and oxidizing conditions to treat effluent variations common to textile mills.

1.2 Problem statement

Treatment of textile wastewater using biological way is environmental friendly as it produces minimal concentrated chemical sludge and toxic intermediates while requiring low operational and maintenance cost. In particular, treatment of textile wastewater using granules in sequencing batch reactor is becoming widely used. This is due to the system retaining high density of biomass that is able to withstand high organic loading along and has high tolerance towards the complex and toxic composition of textile wastewater. Moreover, the development of granules for wastewater treatment in local textile industry generally involved the use of synthetic wastewater containing single or several combinations of dyes. Granules development using real textile wastewater, where the composition is more complex and toxic, on the other hand, was less reported. Consequently, even though majority of textiles plants have set up the remediation facilities, the effluents discharged did not comply with the discharge limit set by local Department of Environment (DOE) (2009) especially on colour and COD removal. Since the granules developed using single or several combination of dyes were not efficient in real textile wastewater treatment, there is a need to develop granules using real textile wastewater. With these high levels of active biomass with broad microbial diversity within the granular system, improvement on the treatment capacity and efficiency with higher bacterial tolerance can be achieved (McHugh *et al.*, 2004). Therefore, a consortium of dye degrading bacteria from RAMATEX textile wastewater plant was used as inoculums to develop granules in sterile real textile wastewater effluent to meet the Standard B of Environmental Quality (Industry Effluents) Regulations 2009 (PU (A) 434) with colour less than 200 ADMI and COD less than 250 mg L^{-1}. Moreover, the biological development using granules has also shown great potential in textile wastewater treatment as the construction of relatively small footprint treatment plant is beneficial for small to intermediate scale industries.

1.3 Objectives

In view of the above problems related to current textile wastewater treatment, research objectives were focused on granulation process implementing novel bacterial consortium and raw textile wastewater. The specific research objectives are given as follows:

i. To isolate, select and identify bacteria able to treat raw textile wastewater treatment.
ii. To develop and characterise the physical properties of granules developed from sterile raw textile wastewater using selected mix culture of bacteria able to treat raw textile wastewater in a sequencing batch reactor system.
iii. To determine the colour and COD removal of developed granules in raw textile wastewater treatment.

1.4 Scope of study

The study involves the development of granules using lab-scale sequencing batch reactor (SBR) system under intermittent facultative anaerobic and aerobic phase with hydraulic retention time (HRT) of 6 hours. A glass column reactor with working volume of 1.5 L was utilised for granulation. The seed sludge used was obtained from the textile wastewater treatment plant in Johor and sterilized before transferring into the column. For the development of biogranules, the inoculums were prepared by growing the selected decolourising bacteria as a mix culture in 1:1:1:1 ratio using sterilized textile wastewater under intermittent facultative anaerobic and aerobic phase. The selected decolourising bacteria consisted of four inoculums; two isolated from local textile wastewater plant while another two obtained from UTM microbiology lab stock cultures. The SBR reactor functions in sequential platform of ‘fill’, ‘react’, ‘settle’, and ‘decant’ with volumetric exchange ratio (VER) of 50% and aeration through reactor foundation. Acclimatized microbes with sterilized textile wastewater were added during the filling stage of every cycle. Granules were developed over a period of time during which samples of granules were collected and examined in terms of their morphology and physical characteristics such as mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), settling velocity and physical strength test. Sequentially, the formed granules were tested for performances in real textile wastewater treatment in terms of colour and COD removal.

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